Supplementary Table 1

	Puro-PLA	**FUNCAT-PLA**
Incorporation	Fast	Slow
	No activation required	Uptake by amino acid transporter and activation by MetRS needed
	Competition with all activated tRNAs	Competition with methionine
	Labeling in full conditioned medium	Labeling in methionine-free medium
	No starvation necessary	Methionine starvation conditions promote labeling (either with pre-starvation or at least during incubation)
	C-terminal incorporation, possible at any site but limited to one Puromycin per protein	Only replacement of methionine residues possible, more than one methionine replacement possible per protein
Protein	Full labeling (= one puromycin per protein) would lead to protein synthesis block	Full labeling in theory possible
	Truncated, premature termination of labeled protein	Full length protein with small bio-orthogonal groups
	Enhanced degradation/ turnover of truncated proteins expected, non-physiological protein fate	Physiological fate
Method characteristics	Fast, sensitive	Lag phase, especially when used without methionine starvation, less sensitive than Puro-PLA
	Short labeling, unlikely to influence short term physiology	Labeling conditions might impact short term physiology, but also see Supplementary Figure 7
	Puro antibodies needed N-terminal POI antibodies are predicted to work better than C-terminal	Biotin antibodies needed
		Additional step required (biotin click)
	Estimating intra- vs intermolecular detection with N-/C-term antibody against POI possible	Modification by direct click of a PLA oligo possible

Recommended method use	Short term measurements Site of synthesis Synthesis rate at a time point	Long term measurements Turnover Half life Distribution changes Synthesis in time interval